

Amendment to the Specification

Please replace the paragraph bridging pages 29 and 30 with the following:

(Amended) (60/263,668) The following vectors may be designed to optimize protein expression, purification and production of proteins with the same amino acid composition as in human insulin.

- a) Using tobacco plants, Eibl (1999) demonstrated, *in vivo*, the differences in translation efficiency and mRNA stability of a GUS reporter gene due to various 5' and 3' untranslated regions (UTR's). This already described systematic transcription and translation analysis can be used in a practical endeavor of insulin production. Consistent with Eibl's (1999) data for increased translation efficiency and mRNA stability, the psbA 5' UTR can be used in addition with the psbA 3' UTR already in use. The 200 bp tobacco chloroplast DNA fragment containing 5' psbA UTR may be amplified by PCR using tobacco chloroplast DNA as template. This fragment may be cloned directly in the pLD vector multiple cloning site downstream of the promoter and the aadA gene. The cloned may be exactly the same as in the psbA gene. (Update "Human Insulin") We have cloned the 5'untranslated region of the tobacco psbA gene including the promoter (5'UTR), shown in Figure 32. We performed PCR using the primers CCGTCGACGTAGAGAAGTCCGTATT SEQ. ID. NO.:4 and GCCCATGGTAAAATCTTGGTTTATTTA, which resulted in a 200 base pair product, as expected. We inserted this PCR product into a TA cloning vector. Since restriction enzyme sites were not available to subclone the 5'UTR immediately upstream of the gene coding for the CTB-proinsulin fusion protein, we used the "SOEing" PCR technique to create the DNA sequence with the 5'UTR immediately upstream of the CTB-proinsulin gene (Figure 33). The products of this PCR include both the 5'UTR (200bp) and the gene for CTB-proinsulin (600bp) as additional products as well as the desired 5'UTR CTB-proinsulin (5CP) at 800 bp. 5CP was eluted and then inserted into the TA cloning vector where DNA sequencing was performed to confirm accuracy of nucleotide sequence before it was subcloned into the pLD vector.